B. Number of seed capsules per plant

Line	Wild-type	AtCKX1-48	AtCKX1-50	AtCKX2-38	AtCKX2-40
Number of capsules	83.33 ± 5.13	2.00 ± 1.00	2.60 ± 1.67	4.30 ± 2.58	n.d.

Experimental: Number of seed capsules was determined at least from 5 different plants. Please note that these plants were grown under green house conditions during winter time. This affects negatively the number of flowers that are formed, in particular in the transgenic clones. However, the general picture that they form a reduced number of flowers is correct. n.d., not determined

C. Seed yield / capsule (mg)

Line	Wild-type	AtCKX1-48	AtCKX1-50	AtCKX2-38	AtCKX2-40
Seed/capsule (mg)	87.41 ± 28.75	23.83 ± 13.36	61.8 ± 40.66	46.98 ± 29.30	n.d.

Experimental: Seed yield was determined for at least 12 seed capsules. The size of seed capsules was very variable, hence the large standard deviations. n.d., not determined

D. Weight of 100 seeds (mg)

Line	Wild-type	AtCKX1-48	AtCKX1-50	AtCKX2-38	AtCKX2-40
Seeds weight (mg)	9.73 ± 0.44	10.70 ± 1.60	9.54 ± 0.94	10.16 ± 0.47	n.d.

Experimental: *The seed biomass was determined as the weight of 100 seed from at least 5 different seed capsules. n.d., not determined*

3.2 In Arabidopsis:

The following morphometric data were obtained for *AtCKX2* transgenics:

Root development

A. Total length of the root system

Line	Wild-type	AtCKX2-2	AtCKX2-5
Length (mm)	32.5	50.6	48.5

B. Primary root length

Line	Wild-type	AtCKX2-2	AtCKX2-5
Length (mm)	32.3 ± 3.8	30.7 ± 4.8	31.6 ± 6.8

C. Lateral roots length

Line	Wild-type	AtCKX2-2	AtCKX2-5
Length (mm)	0.2 ± 0.4	5.5 ± 9.0	1.9 ± 2.5

D. Adventitious roots length

Line	Wild-type	AtCKX2-2	AtCKX2-5
Length (mm)	0.03 ± 0.18	14.4 ± 10.2	14.9 ± 9.1

E. Number of lateral roots (LR)

Line	Wild-type	AtCKX2-2	AtCKX2-5
Number of LR	0.3 ± 0.5	2.9 ± 2.3	1.9 ± 1.0

F. Number of adventitious roots (AR)

Line	Wild-type	AtCKX2-2	AtCKX2-5
Number of AR	0.03 ± 0.18	1.8 ± 0.9	1.8 ± 1.0

Experimental: Measurements were carried out on plants 8 d.a.g. in vitro on MS medium. At least 17 plants per line were scored.

Shoot development

Leaf surface

Line	Wild-type	AtCKX2-2 T2 heterozygous plants	AtCKX2-5 T2 heterozygous plants	AtCKX2-9 T2 heterozygous plants
Leaf surface (cm^2)	21.16 ± 1.73	8.20 ± 2.35	8.22 ± 0.55	7.72 ± 0.85

Experimental: Leaf surface area of main rosette leaves formed after 30 days after germination was measured. 3 plants per clone were analyzed.

Reproductive development

Onset of flowering

Line	Wild-type	AtCKX1-11 T3 heterozygous plants	AtCKX2-2 T2 heterozygous plants	AtCKX2-5 T2 heterozygous plants
Flowering time (DAG)	43.6 ± 5.8	69.7 ± 9.4	51.2 ± 4.1	45.1 ± 6.9

Experimental: Plants were grown under greenhouse condition. At least 13 plants per clone were analyzed. DAG = days after germination.

Conclusion: Arabidopsis *AtCKX2* transgenics had reduced leaf biomass and a dwarfing phenotype similar to *AtCKX1* transgenics (compare Figure 5 with Figure 4 F). The total root system was also enlarged in *AtCKX2* transgenic Arabidopsis. The total root length is increased approximately 50% in *AtCKX2* transgenics. The *AtCKX1* transgenics have longer primary roots, more side roots and form more adventitious roots. *AtCKX2* transgenics lack the enhanced growth of the primary root but form more side roots and lateral roots than WT.

Summary:

The phenotypes observed for *AtCKX2* transgenics were very similar but not identical to the *AtCKX1* transgenics, which in turn were very similar but not identical to the results obtained for the tobacco transgenics. This confirms the general nature of the consequences of a reduced cytokinin content in these two plant species and therefore, similar phenotypes can be expected in other plant species as well. The main difference between tobacco and Arabidopsis is the lack of enhanced primary root growth in *AtCKX2* overexpressing plants.

Example 5. Transgenic plants overexpressing *AtCKX3* showed increased cytokinin oxidase activity and altered plant morphology

1. Description of the cloning process

The following primers were used to PCR amplify the *AtCKX3* gene from *Arabidopsis thaliana*, accession Columbia (non-homologous sequences used for cloning are in lower case):

Sequence of 5´ primer: gcggtaccTTCATTGATAAGAATCAAGCTATTCA (SEQ ID NO:17)

Sequence of 3´ primer: gcggtaccCAAAGTGGTGAGAACGACTAACA (SEQ ID NO:18)

A 3397-bp PCR fragment, produced by this PCR amplification, was inserted in the KpnI site of pBluescript. The insert was sequenced to confirm that the PCR product has no sequence changes as compared to the gene. The KpnI/KpnI fragment of this vector was subcloned in the KpnI site downstream of a modified CaMV 35S promoter (carrying three tetracycline operator sequences) in the binary vector pBinHyg-Tx (Gatz *et al.*, 1992). The resulting construct was introduced into tobacco and *Arabidopsis thaliana* through *Agrobacterium*-mediated transformation, using standard transformation protocols.

2. Molecular analysis of the transgenic lines

Several transgenic tobacco lines were identified that synthesize the *AtCKX3* transcript at high levels (Fig 11 A.). Transgenic tobacco lines expressing *AtCKX3* transcript also showed increased cytokinin oxidase activity. This is exemplified for three plants in Table 8. This proves that the *AtCKX3* gene encodes a protein with cytokinin oxidase activity.

Table 8. Cytokinin oxidase activity in *AtCKX4* transgenic plant tissues

Sample		
Plant species and tissue	**Plant line**	**Cytokinin oxidase activity (nmol Ade/mg protein.h)**
tobacco leaves	SNN wild-type	0.011
	CKX3-SNN-3	0.049
	CKX3-SNN-6	0.053
	CKX3-SNN-21	0.05

3. Plant phenotypic analysis

The phenotypes generated by overexpression of the *AtCKX3* gene in tobacco and *Arabidopsis* were basically similar as those of *AtCKX1* and *AtCKX2* expressing plants, i.e. enhanced rooting and dwarfing. However, overexpression of the *AtCKX3* gene in tobacco resulted in a stronger phenotype compared to *AtCKX2*. In this sense *AtCKX3* overexpression was more similar to *AtCKX1* overexpression.

<u>Example 6. Transgenic plants overexpressing *AtCKX4* showed increased cytokinin oxidase activity and altered plant morphology</u>

1. Description of the cloning process

The following primers were used to PCR amplify the AtCKX4 gene from *Arabidopsis thaliana*, accession Columbia (non-homologous sequences used for cloning are in lower case):

Sequence of 5´ primer: gcggtaccCCCATTAACCTACCCGTTTG (SEQ ID NO:19)

Sequence of 3´ primer: gcggtaccAGACGATGAACGTACTTGTCTGTA (SEQ ID NO:20)

A 2890-bp PCR fragment, produced by this PCR amplification, was inserted in the KpnI site of pBluescript. The insert was sequenced to confirm that the PCR product has no sequence changes as compared to the gene. The KpnI/KpnI fragment of this vector was subcloned in the KpnI site downstream of a modified CaMV 35S promoter (carrying three tetracycline operator sequences) in the binary vector pBinHyg-Tx (Gatz *et al.*, 1992). The resulting construct was introduced into tobacco and *Arabidopsis thaliana* through *Agrobacterium*-mediated transformation, using standard transformation protocols.

2. Molecular analysis of the transgenic lines

Several transgenic tobacco lines synthesized the *AtCKX4* transcript at high levels (Fig 11 B.). Transgenic lines expressing *AtCKX4* transcript also showed increased cytokinin oxidase activity. This is exemplified for 3 A*rabidopsis* and 3 tobacco lines in Table 9. This result proves that the *AtCKX4* gene encodes a protein with cytokinin oxidase activity.

Table 9. Cytokinin oxidase activity in *AtCKX4* transgenic plant tissues

Sample		
Plant species and tissue	**Plant line**	**Cytokinin oxidase activity (nmol Ade/mg protein.h)**
Arabidopsis callus	Col-0 wild-type	0.037
	CKX4-37	0.244
	CKX4-40	0.258
	CKX4-41	0.320
tobacco leaves	SNN wild-type	0.011
	CKX4-SNN-3	0.089
	CKX4-SNN-18	0.085
	CKX4-SNN-27	0.096

Overall, the data showed that the apparent K_m values for the four cytokinin oxidases were in the range of 0.2 to 9.5 μM with iP as substrate, which further

demonstrates that the proteins encoded by *AtCKX1* through *4* are indeed cytokinin oxidase enzymes as disclosed herein.

3. Plant phenotypic analysis

The phenotypes generated by overexpression of the *AtCKX4* gene in tobacco and *Arabidopsis* were basically similar as those of *AtCKX1* and *AtCKX2* expressing plants, i.e. enhanced rooting, reduced apical dominance, dwarfing and yellowing of intercostal regions in older leaves of tobacco. An additional phenotype in tobacco was lanceolate leaves (altered length-to-width ratio).

General observations of *AtCKX* overexpressing tobacco plants

Overall, the phenotypic analysis demonstrated that *AtCKX* gene overexpression caused drastic developmental alterations in the plant shoot and root system in tobacco, including enhanced development of the root system and dwarfing of the aerial plant part. Other effects such as altered leaf senescence, formation of adventitious root on stems, and others were also observed as disclosed herein. The alterations were very similar, but not identical, for the different genes. In tobacco, *AtCKX1* and *AtCKX3* overexpressors were alike as were *AtCKX2* and *AtCKX4*. Generally, the two former showed higher expression of the traits, particularly in the shoot. Therefore, a particular cytokinin oxidase gene may be preferred for achieving the phenotypes that are described in the embodiments of this invention.

Example 7. Cloning of the *AtCKX5* gene

The following primers were used to PCR amplify the AtCKX5 gene from *Arabidopsis thaliana*, accession Columbia (non-homologous sequences used for cloning are in lower case):

Sequence of 5´ primer: ggggtaccTTGATGAATCGTGAA*ATG*AC (SEQ ID NO:21)

Sequence of 3´ primer: ggggtaccCTTTCCTCTTGGTTTTGTCCTGT (SEQ ID NO:22)

The sequence of the 5' primer includes the two potential start codons of the AtCKX5 protein, the most 5' start codon is underlined and a second ATG is indicated in italics.

A 2843-bp PCR fragment, produced by this PCR amplification, was inserted as a blunt-end product in pCR-Blunt II-TOPO cloning vector (Invitrogen).

Example 8. Cloning of the *AtCKX6* gene

The following primers were used to PCR amplify the AtCKX6 gene from *Arabidopsis thaliana*, accession Columbia (non-homologous sequences used for cloning are in lower case):

Sequence of 5´ primer: gctctagaTCAGGAAAAGAACCATGCTTATAG (SEQ ID NO:23)

Sequence of 3´ primer: gctctagaTCATGAGTATGAGACTGCCTTTTG (SEQ ID NO:24)

A 1949-bp PCR fragment, produced by this PCR amplification, was inserted as a blunt-end product in pCR-Blunt II-TOPO cloning vector (Invitrogen).

Example 9. Tobacco seedling growth test demonstrated early vigor of *AtCKX* transgenics

Seeds of *AtCKX1-50* and *AtCKX2-38* overexpressing transgenics and WT tobacco were sown *in vitro* on MS medium, brought to culture room 4 days after cold treatment and germinated after 6 days. Observations on seedling growth were made 10 days after germination (see also Figure 8C) and are summarized below. At least 20 individuals were scored per clone. Similar data have been obtained in two other experiments.

A. Total length of the root system

Line	Wild-type	AtCKX1-50	AtCKX2-38
Length (mm)	61.1	122.0	106.5

B. Primary root length

Line	Wild-type	AtCKX1-50	AtCKX2-38
Length (mm)	32.3 ± 2.6	50.8 ± 4.5	52.4 ± 4.8

C. Lateral roots length

Line	Wild-type	AtCKX1-50	AtCKX2-38
Length (mm)	9.8 ± 5.5	18.0 ± 8.1	13.0 ± 6.0

D. Adventitious roots length

Line	Wild-type	AtCKX1-50	AtCKX2-38
Length (mm)	19.0 ± 5.0	53.0 ± 12.0	42.0 ± 9.8

E. Number of lateral roots (LR)

Line	Wild-type	AtCKX1-50	AtCKX2-38
Number of LR	1.9 ± 0.9	6.5 ± 2.2	5.6 ± 2.0

F. Number of adventitious roots (AR)

Line	Wild-type	AtCKX1-50	AtCKX2-38
Number of AR	2.2 ± 0.6	3.5 ± 0.9	3.6 ± 1.3

AtCKX1 and *AtCKX2* plants, general observations:

Seedlings of *AtCKX1* and *AtCKX2* overexpressing tobacco plants had 60% more adventitious roots and three times more lateral roots than untransformed control plants 10 days after germination. The length of the primary root was increased by about 70%. This – together with more and longer side roots and secondary roots – resulted in a 70-100% increase in total root length. These results showed that overexpression of cytokinin oxidase enhances the growth and development of both the main root and the adventitious roots, resulting in early vigor.

Example 10. Histological analysis of altered plant morphology in *AtCKX1* overexpressing tobacco plants

Microscopic analysis of different tissues revealed that the morphological changes in *AtCKX* transgenics are reflected by distinct changes in cell number and rate of cell formation (see Figure 10). The shoot apical meristem (SAM) of *AtCKX1* transgenics was smaller than in wild type and fewer cells occupy the space between the central zone and the peripheral zone of lateral organ formation, but the cells were of the same size (Figure 10 A). The reduced cell number and size of the SAM as a consequence of a reduced cytokinin content indicates that cytokinins have a role in the control of SAM proliferation. No obvious changes in the differentiation pattern occurred, suggesting that the spatial organization of the differentiation zones in the SAM is largely independent from cell number and from the local cytokinin concentration. The overall tissue pattern of leaves in cytokinin oxidase overexpressors was unchanged. However, the size of the phloem and xylem was significantly reduced (Figure 10 B). By contrast, the average cell size of leaf parenchyma and epidermal cells was increased four- to fivefold (Figure 10 C, D). New cells of *AtCKX1* transgenics are formed at 3-4% of the rate of wild type leaves and final leaf cell number was estimated to be in the range of 5-6% of wild type. This indicates an absolute requirement for cytokinins in leaves to maintain the cell division cycle. Neither cell size nor cell form of floral organs was altered and seed yield per capsule was similar in wild type and *AtCKX* transgenic plants. The cell population of root meristems of *AtCKX1* transgenic plants was enlarged approximately 4-fold and the cell numbers in both the central and lateral columnella were enhanced (Figure 10 E, F). The final root diameter was increased by 60% due to an increased diameter of all types of root cells. The radial root patterns was identical in wild type and transgenics, with the exception that frequently a fourth layer of cortex cells was noted in transgenic roots (Figure 10 G). The increased cell number and the slightly reduced cell length indicates that the enhanced root growth is due to an increased number of cycling cells rather than increased cell growth. In the presence of lowered cytokinin content, root meristem cells must undergo additional rounds of mitosis before they leave the meristem and start to elongate. The exit from the meristem is therefore regulated by a mechanism that is sensitive to cytokinins. Apparently, cytokinins have a negative regulatory role in the root meristem and wild type cytokinin concentrations are inhibitory to the development of a maximal root

system. Therefore, reducing the level of active cytokinins by overexpressing cytokinin oxidases stimulates root development, which results in an increase in the size of the root with more lateral and adventitious roots as compared to WT plants.

Example 11. *AtCKX1* and *AtCKX2*- overexpressing tobacco plants had a reduced cytokinin content.

Among the 16 different cytokinin metabolites that were measured, the greatest change occurred in the iP-type cytokinins in *AtCKX2* overexpressers (Table 10): the overall decrease in the content of iP-type cytokinins is more pronounced in *AtCKX2* expressing plants than in *AtCKX1* transgenics. *AtCKX1* transgenics showed a stronger phenotype in the shoot. It is not known which cytokinin metabolite is relevant for the different traits that were analysed. It may be that different cytokinin forms play different roles in the various development processes. Smaller alterations were noted for Z-type cytokinins, which could be due to a different accessibility of the substrate or a lower substrate specificity of the protein. The total content of iP and Z metabolites in individual transgenic clones was between 31% and 63% of wild type. The cytokinin reserve pool of *O*-glucosides was also lowered in the transgenics (Table 10). The concentration of *N*-glucosides and DHZ-type cytokinins was very low and was not or only marginally, altered in transgenic seedlings (data not shown).

Table 10. Cytokinin content of *AtCKX* transgenic plants. Cytokinin extraction, immunopurification, HPLC separation and quantification by ELISA methods was carried out as described by Faiss et al., 1997. Three independently pooled samples of approximately 100 two week old seedlings (2.5 g per sample) were analysed for each clone. Concentrations are in pmol x g fresh weight^{-1}. Abbreviations: iP, N^6-(Δ^2isopentenyl)adenine; iPR, N^6-(Δ^2isopentenyl)adenine riboside; iPRP, N^6-(Δ^2isopentenyl)adenine riboside 5´-monophosphate; Z, *trans*-zeatin; ZR, zeatin riboside; ZRP, zeatin riboside 5´-monophosphate; ZOG, zeatin *O*-glucoside; ZROG, zeatin riboside *O*-glucoside.

Line	WT	AtCKX1-2		AtCKX1-28		AtCKX2-38		AtCKX2-40	
Cytokinin meta-bolite	Concen-tration	Concen-tration	% of WT	Concen-tration	% of WT	Concen-tration	% of WT	Concen-tration	% of WT
iP	5.90 ± 1.80	4.76 ± 0.82	81	4.94 ± 2.62	84	1.82 ± 0.44	31	2.85 ± 0.62	48
iPR	2.36 ± 0.74	1.53 ± 0.14	65	0.75 ± 0.27	32	0.55 ± 0.39	23	0.89 ± 0.07	38
iPRP	3.32 ± 0.73	0.87 ± 0.26	26	1.12 ± 0.13	34	0.80 ± 0.48	24	1.68 ± 0.45	51
Z	0.24 ± 0.06	0.17 ± 0.02	71	0.22 ± 0.03	92	0.21 ± 0.06	88	0.22 ± 0.02	92
ZR	0.60 ± 0.13	0.32 ± 0.12	53	0.34 ± 0.03	57	0.34 ± 0.15	57	0.32 ± 0.05	53
ZRP	0.39 ± 0.17	0.42 ± 0.11	107	0.28 ± 0.15	72	0.06 ± 0.01	15	0.17 ± 0.06	44
ZOG	0.46 ± 0.20	0.32 ± 0.09	70	0.26 ± 0.13	57	0.20 ± 0.07	43	0.12 ± 0.02	26
ZROG	0.48 ± 0.17	0.30 ± 0.06	63	0.47 ± 0.02	98	0.23 ± 0.05	48	0.30 ± 0.13	63
Total	13.75	8.69	63	8.38	61	4.21	31	6.55	48

Example 12. Grafting experiments showed that dwarfing and enhanced root development due to *AtCKX* overexpression is confined to transgenic tissues

To investigate which phenotypic effects of cytokinin oxidase overexpression are restricted to expressing tissues, i.e. are cell- or organ-autonomous traits, grafting experiments were performed. Reciprocal grafts were made between an *AtCKX2* transgenic tobacco plant and a WT tobacco. The transgenic plant used in this experiment was AtCKX2-38, which displayed a strong phenotype characterized by enhanced root growth and reduced development of the aerial plant parts. As

described in Example 3 through 6, these were two important phenotypes that resulted from cytokinin oxidase overexpression in tobacco and arabidopsis.

Plants were about 15 cm tall when grafted and the graft junction was about 10 cm above the soil. Figure 12 shows plants 15 weeks after grafting. The main results were that : (i) the aerial phenotype of a WT scion grafted on a transgenic rootstock was similar to the WT control graft (= WT scion on WT rootstock). Importantly, this showed that overexpression of the *AtCKX2* transgene in the rootstock did not induce dwarfing of the non-transgenic aerial parts of the plant (see Figure 12 A). Improved root growth of the transgenic rootstock was maintained, indicating that improved root growth of *AtCKX* transgenics is autonomous and does not depend on an *AtCKX* transgenic shoot (Figure 12 C). Interestingly, the WT scions grafted on the transgenic rootstocks looked healthier and were better developed. Notably, senescence of the basal leaves was retarded in these plants (see Figure 12 A); (ii) the transgenic scion grafted on the WT rootstock looked similar to the aerial part of the transgenic plant from which it was derived, i.e. the shoot dwarfing phenotype is also autonomous and not dependent on the improved root growth (see Figure 12 B).

In addition to the above-mentioned better appearance of WT shoots grafted on a transgenic rootstock, the formation of adventitious roots on the basal part of WT shoots was noted (Figure 12 D, right plant). Formation of adventitious roots also occurred on the stem of *AtCKX* transgenics but not on stems of WT control grafts (Figure 12 D, left plant) and therefore seems to be a non-autonomous trait.

In summary, it is disclosed in this invention that enhanced root formation and dwarfing of the shoot in *AtCKX* overexpressing tobacco are autonomous traits and can be uncoupled by grafting procedures. Surprisingly, grafting of a WT scion on an *AtCKX* transgenic rootstock resulted in more vigorously growing plants and retardation of leaf senescence.

As an alternative to grafting, tissue-specific promoters could be used for uncoupling the autonomous phenotypic effects of cytokinin overexpression. Therefore, it is disclosed in this invention that cytokinin oxidase overexpression in

a tissue specific manner can be used to alter the morphology of a plant such as the shoot or root system.

Example 13. Expression of an *AtCKX* gene under a root-specific promoter in transgenic plants leads to increased root production

An *AtCKX* gene (see example 4) is cloned under control of the root clavata homolog promoter of *Arabidopsis* (SEQ ID NO: 36) , which is a promoter that drives root-specific expression. Other root-specific promoters may also be used for the purpose of this invention. See Table 5 for exemplary root-specific promoters.

Transgenic plants expressing the *AtCKX* gene specifically in the roots show increased root production without negatively affecting growth and development of the aerial parts of the plant. Positive effects on leaf senescence and growth of aerial plant parts are observed.

Example 14. Suppression of an *AtCKX* gene under a senescence-induced promoter in transgenic plants leads to delayed leaf senescence and enhanced seed yield.

A chimeric gene construct derived from an *AtCKX* gene and designed to suppress expression of endogenous cytokinin oxidase gene(s) is cloned under control of a senescence-induced promoter. For example, promoters derived from senescence-associated genes (SAG) such as the SAG12 promoter can be used (Quirino et al., 2000). Transgenic plants suppressing endogenous cytokinin oxidase gene(s) specifically in senescing leaves show delayed leaf senescence and higher seed yield without negatively affecting the morphology and growth and development of the plant.

Example 15. Overexpression of an *AtCKX* gene in the female reproductive organs leads to parthenocarpic fruit development

The open reading frame of an *AtCKX* gene is cloned under control of a promoter that confers overexpression in the female reproductive organs such as for example the DefH9 promoter from *Antirrhinum majus* or one of its homologues, which

have high expression specificity in the placenta and ovules. Transgenic plants with enhanced cytokinin oxidase activity in these tissues show parthenocarpic fruit development.

Example 16. Overexpression of AtCKX genes result in increased seed and cotyledon size

Transgenic *Arabidopsis thaliana* plants that overexpress cytokinin oxidase (*AtCKX*) genes under control of the 35S promoter as described supra. Transgenic plants, in particular those expressing the *AtCKX1* and *AtCKX3* genes, developed seeds with increased size which was almost entirely due to an enlarged embryo. Details of the seed, embryo and early postembryonic phenotypes are shown in Figures 13 A through 13E. Table 11 shows seed weight of wild type and two independent clones for each of the four investigated AtCKX genes. Average weight was obtained by analysing five different batches of 200 seeds for each clone. A quantitative evaluation showed that the seed weight of *AtCKX1* and *AtCKX3* expressing clones was app. 1.8-2.3-fold higher than in wild type. Gain of weight for seeds of *AtCKX2* and *AtCKX4* expressing lines was in the range of 10-25% (Table 11 and Fig. 14).

The increases in size and weight for seeds, embryos, and cotyledons are unexpected as a reduced cytokinin content would have been expected to be associated with a reduced organ growth. One possible reason for the increases in seed, embryo, and cotyledon size is a previously unknown negative regulatory function of cytokinins in these storage organs. A negative regulatory functions of cytokinins in the control of organ growth is so far only known from roots (Werner et al. 2001). We propose, therefore, that localized expression of cytokinin oxidase genes in tissues where growth is negatively regulated by cytokinins leads to enhanced growth of this tissue. For example, localized expression of *CKX* genes during cotyledon development likely leads to enhanced growth of cotyledons and in species with cotyledons as storage organs, to enhanced yield and to an enhanced growth performance of seedlings. Total number of seeds is lowered in *AtCKX1* and *AtCKX3* expressers. There have been no previous reports however, of lower seed number in *Arabidopsis* being linked to an increase in size.

TABLE 11

	WT	CKX1-11-7	CKX1-15-1	CKX2-2-4	CKX2-9-3	CKX3-9-4	CKX3-12-13	CKX4-37-2	CKX4-41-7
Seed Weight	0.0158±0.0009	0.0372±0.0015	0.0352±0.0023	0.0201±0.0017	0.0180±0.0001	0.0340±0.0027	0.0280±0.0027	0.0185±0.0004	0.0179±0.0007
% of WT	100	235.5	222.6	126.7	113.7	215.0	176.7	116.8	112.7

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